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## *In Vitro* Direct Shoot Organogenesis from Leaf Explants of *Solanum nigrum* (L.) – An Important Antiulcer Medicinal Plant

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Article Info	Summary
<b>Article History</b> Received : 30-02-2011 Revised : 23-03-2011 Accepted : 29-03-2011	<p>In the present study, a protocol for direct shoot organogenesis was developed from both field grown and <i>in vitro</i> derived leaf explants. The regeneration of shoots from leaf was found to vary with varying concentrations of BAP. The combination of BA (2.0 mg/L) and IAA (0.5 mg/L) produced maximum number of shoots (32.8) from leaf explants of field grown <i>Solanum nigrum</i> plants. Where as in <i>in vitro</i> derived leaf explants maximum number of shoots (38.0) was obtained on BA (3.0 mg/L) and IAA (0.5 mg/L) combination. All the <i>in vitro</i> raised shoots with a length of 3-5 cm were transferred to rooting medium supplemented with different concentrations of auxins such as IBA, NAA and IAA (0.25 – 1.0 mg/l). The best rooting response was observed on 0.5mg/l IBA. The well rooted plantlets were transferred to polybags containing soil + vermiculite in 1: 1 ratio for hardening. Finally the hardened plantlets were transferred to field conditions for maximum survivability. From the obtained results it is evident that leaf material is the best source for inducing maximum number of multiple shoots via direct shoot regeneration in <i>Solanum nigrum</i> plants.</p>
<b>*Corresponding Author</b> Tel : +91 877 2260386 Fax : +91-8570278209 Email: <a href="mailto:challagundlav@yahoo.co.in">challagundlav@yahoo.co.in</a> <a href="mailto:thulasimsreedhar@gmail.com">thulasimsreedhar@gmail.com</a>	<p><b>Key Words:</b> Leaf explants, Plant regeneration, BA, Kn, NAA, IAA, GA<sub>3</sub>, <i>Solanum nigrum</i> (L.)  <b>Abbreviations:</b> BA- 6-benzyladenine; Kn- kinetin; NAA- α-naphthalene acetic acid; IAA- indole-3-acetic acid, IBA – Indole-3-butyric acid; GA<sub>3</sub> – Gibberellic acid</p>

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### Introduction

*Solanum nigrum* is commonly known as 'kamanchi' in Sanskrit 'makoi' in Hindi 'kassipandu' in Telugu, 'black knight shade' in English and is belongs to the family solanaceae. It is grown in dry parts of India up to an elevation of 2,100 mts. The hexaploid solanum however occurs mostly in temperate parts and are rarely in warmer regions [5]. And this annual herbaceous plant can grow up to 100 cm height. The stem may be smooth or bear small hairs (trichomes). The flowers usually white in colour have five regular petals and are up to 0.8 cm. wide, recurved when aged and surround bright yellow anthers. The leaves are alternative and are ovate with irregularly toothed wavy margin and can reach upto 10 cm in length and 5cm in width. The fruit is a round fleshy berry up to 2 cm in diameter and purple black when ripe, the seeds are dark brown and numerous. In India, another strain is found with berries that turn red when ripe. It is well suited for all type of cultivated lands.

### Culinary usage

In India *Solanum nigrum* is generally grown in pots as a homestead garden plant and is commonly used as a leafy vegetable in preparing dishes and soups in Northern Tamilnadu, Southern Andhra Pradesh and Southern Karnataka of India. In north India, the boiled extracts of leaves and berries are also used to alleviate the patient's discomfort in liver related ailments, including jaundice.

### Medicinal properties

The plant has a long history of medicinal usage, dating back to ancient Greece. It is an important herbaceous medicinal plant, generally used in traditional and folklore medicines. The herb is antiseptic, antidiarrhetic and diuretic used in the treatment of cardiac, skin diseases, psoriasis, herpesvirus and inflammation of kidney. The leaves, stems and roots are used externally as poultice, wash etc., in the treatment of cancerous soles, boils, leucoderma and wounds [19]. The fruits and leaves have been traditionally used against various nerve disorders [23]. Root bark is laxative, useful in the treatment of neck, burning of throat, inflammation of liver, chronic fever. Berries are bitter, pungent and are useful in the disease of heart, piles and dysentery [14]. It has very important gastric ulcerogenic activities [1]. Recent findings reports that the plant is used as febrifuge and in the treatment of hydrophobia, eye disease. Extracts of the plant are analgesic, antispasmodic, anti-inflammatory and vasodilator [19]. Most important aspect of this medicinal plant is that it contains two important alkaloids solamargin and solasonine which yield solasodine [4] as glycone. Solasodine has embryogenic, teratonic as well as antifungal, antiviral and molluscidal effects [12].

The present investigation was aimed to understand the role of different growth substances on *in vitro* plant regeneration from young leaf explants of *Solanum nigrum*.

## Materials and Methods

### Plant material

Young leaves of *Solanum nigrum* (L.) were collected from two-month-old seed germinated field grown plants growing in nursery of Biotechnology Department, S.V. University, Tirupati, Andhra Pradesh, India.

### Surface sterilization

Explants were washed thoroughly under running tap water to remove traces of dust etc. followed by treatment with 10% teepol or tween -20 for 5 minutes. Then the explants were sterilized in 70% alcohol for a minute, and finally with 0.01% mercuric chloride for 1-2 minutes and washed 3-4 times with sterile double distilled water.

### Culture Medium

The explants were inoculated on MS medium [20] containing 3% sucrose and gelled with 0.8% agar, supplemented with various concentrations of BAP, Kn alone or in combination with IAA or NAA. The pH of the medium was adjusted to 5.8 before gelling with agar and autoclaved for 20 minutes at 121°C and 15 lbs pressure.

### Sub culturing

The cultures were maintained by regular subculture at 4 week intervals on fresh MS medium.

### Culture conditions

The growth room conditions maintained for *in vitro* cultures were 26 ± 2°C and 60-70% relative humidity, light intensity was 3000 lux with 18 hrs day light and 6 hrs dark.

Each experiment was conducted at least thrice with 20 replicates per treatment.

## Results

Growth and organogenesis *in vitro* depends on the interaction between endogenous growth substances and the synthetic growth regulators which may be added to the medium. The regeneration of adventitious shoots from leaf explants was dependent on both auxin and cytokinin in the medium.

### Direct shoot organogenesis from field grown leaf explants

#### Effect of Cytokinins on direct shoot regeneration

Effect of two different cytokinins (BA, Kn) on direct shoot induction was examined. In leaf explants after two weeks of inoculation leaf folding, leaf enlargement were noticed. Shoot bud primordial was emerged along the cutted portions and midrib regions of young leaf explants, later they were developed as shoots. In BAP (0.5-3.0 mg/L) alone supplemented MS medium shoot initiation was observed and the mean number of shoots ranged from 12-20 with mean shoot length of 3-5 cm respectively. The optimum concentration was found to be 2.0 mg/L BAP. The number of multiple shoots decreased with further increase in cytokinin concentration (> 3.0 mg/L) and it promotes callusing. In Kn (0.5 – 3.0 mg/L) alone supplemented MS medium shoot bud initiation was observed and the mean number of shoots ranged from 6-12 with mean shoot length of 6-9 cm. The number of shoots was less compare to BAP supplemented medium but maximum shoot length 9.0 cm was observed (Table-1; Figure-1).

Table-1: *In vitro* direct multiple shoot organogenesis from leaf explants of field grown *Solanum nigrum* plants. Observation: After 4 weeks, values are mean ± S.E. of 20 independent determinants.

MS+						Number of shoots / explants	Length of shoot (cm)
BA	Kn	NAA	IAA	IBA	GA <sub>3</sub>		
0.5	-	-	-	-	-	12.8±0.34	5.4±0.34
1.0	-	-	-	-	-	17.2±0.18	5.0±0.28
2.0	-	-	-	-	-	20.6±0.53	3.6±0.21
3.0	-	-	-	-	-	18.4±0.21	3.2±0.17
-	0.5	-	-	-	-	10.4±0.21	6.3±0.18
-	1.0	-	-	-	-	12.8±0.34	7.0±0.28
-	2.0	-	-	-	-	8.0±0.28	8.2±0.17
-	3.0	-	-	-	-	6.3±0.31	9.0±0.28
1.0	-	0.5	-	-	-	18.6±0.53	6.4±0.21
2.0	-	0.5	-	-	-	24.0±0.28	7.0±0.34
3.0	-	0.5	-	-	-	19.3±0.31	5.6±0.53
-	1.0	0.5	-	-	-	17.8±0.17	9.2±0.18
-	2.0	0.5	-	-	-	16.2±0.50	10.5±0.50
-	3.0	0.5	-	-	-	12.6±0.53	11.0±0.28
1.0	-	-	0.5	-	-	24.0±0.28	7.2±0.18
2.0	-	-	0.5	-	-	32.8±0.34	7.8±0.34
3.0	-	-	0.5	-	-	27.3±0.31	6.6±0.53
-	1.0	-	0.5	-	-	17.6±0.53	9.2±0.18
-	2.0	-	0.5	-	-	15.0±0.28	10.8±0.34
-	3.0	-	0.5	-	-	12.4±0.21	11.4±0.21
1.0	-	-	-	0.5	-	5.4±0.34	4.6±0.53
2.0	-	-	-	0.5	-	6.5±0.50	4.0±0.28
1.0	-	-	-	-	0.5	8.0±0.28	6.2±0.50
2.0	-	-	-	-	1.0	10.0±0.28	8.4±0.34

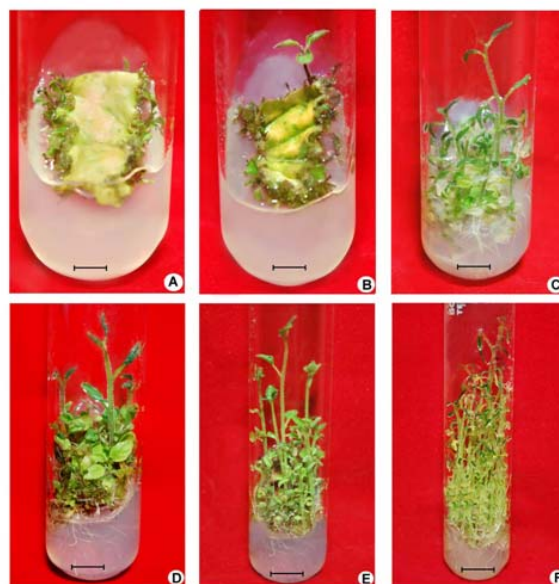


Figure-1: Direct regeneration of shoots from leaf explants of field grown *S. nigrum* plants.

- A) Shoot initiation from leaf explants of field grown plants  
MS + 0.5 mg/L BA (bar 1 cm = 0.65)
- B) Shoot initiation from leaf explants of field grown plants  
MS + 1.0 mg/L BA (bar 1 cm = 0.60)
- C) Shoot initiation from leaf explants of field grown plants  
MS + 2.0 mg/L BA (bar 1 cm = 0.50)
- D) Multiplication of shoots from leaf explants  
MS + 1.0 mg/L BA + IAA (0.5 mg/L) (bar 1 cm = 1.0)
- E, F) Elongated multiple shoots regenerated from leaf explants  
MS + BA (2.0 mg/L) + IAA (0.5 mg/L) (bar 1 cm = 1.1 and 1.2 respectively)

#### **Effect of cytokinin in combination with auxin on direct shoot regeneration**

Growth regulator requirement for direct shoot formation are generally summarized (a cytokinin to auxin ratio of 2-3:1) [10]. The combination of BA (0.5-3.0 mg/L) and NAA, IAA (0.5 mg/L) was studied. In BAP (0.5-3.0 mg/L) and IAA (0.5 mg/L) combination multiple shoots were induced with mean shoot number of 10.0-32.8 and the mean shoot length of 6-11 cm respectively. The highest number of shoots  $32.8 \pm 0.34$  was obtained on BA (2.0 mg/L) and IAA (0.5 mg/L) supplemented MS medium. BAP in combination with NAA induced multiple shoots with mean shoot number of 8.0-24.0 and the mean shoot length of 3.0-7.0 cm respectively. BAP (1.0-2.0) in combination of IBA (0.5 mg/L) induced less number of multiple shoots with mean shoot number of 5.4-6.5 and mean shoot length of 4.0-4.6 cm respectively (Table-1). Among the auxins tested (NAA, IAA and IBA) in combination with BAP natural auxin IAA proved to be better for providing maximum number of shoots.

#### **Effect of Gibberellin GA<sub>3</sub> on direct shoot regeneration**

The combination of BA (1.0-2.0 mg/L) and GA<sub>3</sub> (0.5-1.0 mg/L) were used to test the effect of GA<sub>3</sub> on direct organogenesis and GA<sub>3</sub> promoted shoot elongation. The mean number of shoots ranged from 8.0-10 with mean shoot length of 6.2-8.4 cm (Table-1) respectively. The shoots formed

in GA<sub>3</sub> supplemented MS medium were of healthy with maximum shoot length.

#### **Direct shoot organogenesis from in vitro derived leaf**

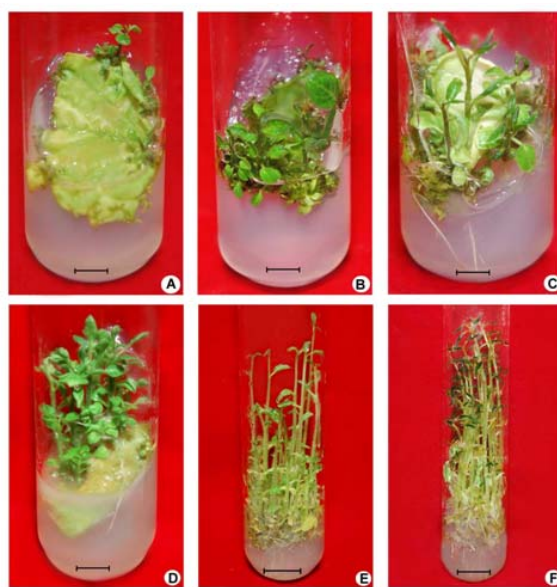
##### **Effect of cytokinin in combination with auxin on direct shoot regeneration from in vitro derived leaf explants**

Effect of cytokinins (BA, Kn) in combination with auxins (NAA or IAA or IBA) on direct shoot induction was examined. BA (0.5-3.0 mg/L) in combination of auxins like NAA, IAA and IBA (0.5 mg/L) were used to produce the direct shoot regeneration from young leaf of *in vitro* regenerated *Solanum nigrum* plants. In leaf explants after two weeks of inoculation leaf foldings, leaf enlargement was noticed. Shoot bud primordial was emerged along the cut portions and midrib regions of leaf explants and later they were developed as shoots.

In BAP and NAA combination the mean number of shoots obtained was 12.0-26.0 with mean shoot length of 4.0-6.0 cm respectively. The highest number of shoots obtained on BA (2.0 mg/L) and NAA (0.5 mg/L). In BA and IAA supplemented medium the mean number of shoots 14.0-38.0 was obtained with mean shoot length of 5.0-8.3 cm respectively. Where as the maximum number of shoots was obtained on BA (2.0 mg/L) and IAA (0.5 mg/L) supplemented medium (Table-2; Figure-2).

Table-2: Direct shoot organogenesis from leaf of *in vitro* regenerated *Solanum nigrum* plants. Observation: After 4 weeks, values are mean  $\pm$  S.E. of 20 independent determinants

MS+						Number of shoots / explants	Length of shoot (cm)
BA	Kn	NAA	IAA	IBA	GA <sub>3</sub>		
0.5	-	0.5	-	-	-	12.5 $\pm$ 0.31	4.0 $\pm$ 0.23
1.0	-	0.5	-	-	-	18.8 $\pm$ 0.20	5.1 $\pm$ 0.25
2.0	-	0.5	-	-	-	26.0 $\pm$ 0.23	5.6 $\pm$ 0.40
3.0	-	0.5	-	-	-	20.0 $\pm$ 0.47	3.8 $\pm$ 0.16
1.0	1.0	0.1	-	-	-	15.0 $\pm$ 0.23	6.0 $\pm$ 0.40
1.5	1.0	0.1	-	-	-	18.1 $\pm$ 0.25	6.5 $\pm$ 0.50
2.0	1.0	0.1	-	-	-	22.0 $\pm$ 0.40	5.0 $\pm$ 0.40
0.5	-	-	0.5	-	-	14.5 $\pm$ 0.31	7.6 $\pm$ 0.42
1.0	-	-	0.5	-	-	26.0 $\pm$ 0.23	8.0 $\pm$ 0.47
2.0	-	-	0.5	-	-	38.0 $\pm$ 0.40	8.3 $\pm$ 0.31
3.0	-	-	0.5	-	-	28.5 $\pm$ 0.31	7.0 $\pm$ 0.40
1.0	1.0	-	0.1	-	-	15.3 $\pm$ 0.45	7.1 $\pm$ 0.25
2.0	1.0	-	0.1	-	-	18.8 $\pm$ 0.20	8.8 $\pm$ 0.18
3.0	1.0	-	0.1	-	-	24.0 $\pm$ 0.23	6.5 $\pm$ 0.31
1.0	1.0	-	-	0.1	-	16.5 $\pm$ 0.50	5.6 $\pm$ 0.42
2.0	1.0	-	-	0.1	-	20.0 $\pm$ 0.40	4.5 $\pm$ 0.50
1.0	-	-	-	-	0.5	14.6 $\pm$ 0.42	8.6 $\pm$ 0.33
2.0	-	-	-	-	1.0	18.0 $\pm$ 0.23	10.0 $\pm$ 0.28

Figure-2: Direct regeneration of shoots from leaf explants of *in vitro* grown *S. nigrum* plants.

- A) Shoot bud initiation from leaf explants  
MS + BA (1.0 mg/L) (bar 1 cm = 0.5)  
B-C) Shoot bud initiation from leaf explants  
MS + BA (2.0 mg/L) (bar 1 cm = 0.65 and 1.1)  
D) Multiplication of shoots from leaf explants  
MS + BA (1.0 mg/L) + NAA (0.5 mg/L) (bar 1 cm = 1.6)  
E-F) Elongated multiple shoots regenerated from leaf explants  
MS + BA (2.0 mg/L) + NAA (0.5 mg/L) (bar 1 cm = 1.2 and 1.3)

#### Effect of two different cytokinins with an auxin on direct shoot regeneration

BA (1.0-2.0 mg/L) and kinetin (1.0 mg/L) in combination of lower concentrations of NAA, IAA (0.1 mg/L) also favoured the shoot formation. The combination of BA-Kn and IAA favoured the shoot formation with mean shoot number of 15.0-22.0 with shoot length of 5.0-6.0 cm respectively. Where as the

combination of BA (1.0-2.0 mg/L) and kinetin (1.0 mg/L) when used with IAA (0.1 mg/L) showed the increased shoot number 15.3-24.0, but maximum number of shoots (24.0) was formed on 2.0 mg/L BAP and 1.0 mg/L Kn with 0.1 mg/L IAA (Table-2). BA (1.0-2.0 mg/L) and kinetin (1.0 mg/L) in combination with IBA (0.1 mg/L) also resulted in the formation of multiple shoots 16.0-20.0 with mean shoot length of 4.5-5.6 cm, but the

number of shoots decreased compared with BA-Kn and IAA, BA-Kn and NAA combinations.

#### Effect of GA<sub>3</sub> on direct shoot regeneration

The combination of BA (1.0-2.0 mg/L) and GA<sub>3</sub> (0.5-1.0 mg/L) were used to test the effect of GA<sub>3</sub> on direct organogenesis. GA<sub>3</sub> promoted shoot elongation and the mean number of shoots ranged from 14.0-18.0 with mean shoot length of 8-10 cm respectively (Table-2).

#### In vitro rooting

The shoots developed *in vitro* were excised from shoot clump and transferred to MS medium supplemented with different concentration of auxins such as IBA, NAA and IAA (0.25-1.0 mg/L). The best rooting response was observed on IBA (0.5 mg/L) supplemented medium, where maximum number of roots (38.2) were obtained followed by NAA (0.5 mg/L), IAA (0.5 mg/L) supplemented medium where 24.1, 17.8 number of roots were induced respectively. The number of roots decreased with further increase in auxin concentration (>0.5mg/L)(Table-3;Figure-3).

Table – 3: Effect of different auxins on *in vitro* rooting. Results are mean  $\pm$  S.E of 20 replicates

Plant growth regulators (mg/l)	Frequency (%)	Mean number of roots	Mean root length (cm)
IBA			
0.25	95	26.0 $\pm$ 0.35	4.2 $\pm$ 0.18
0.5	100	38.2 $\pm$ 0.18	5.0 $\pm$ 0.28
0.75	94	28.0 $\pm$ 0.23	3.6 $\pm$ 0.35
1.0	90	18.4 $\pm$ 0.22	2.3 $\pm$ 0.31
NAA			
0.25	90	18 $\pm$ 0.23	3.4 $\pm$ 0.35
0.5	95	24.1 $\pm$ 0.43	4.5 $\pm$ 0.31
0.75	85	21 $\pm$ 0.56	3.8 $\pm$ 0.17
1.0	74	14.2 $\pm$ 0.18	2.0 $\pm$ 0.28
IAA			
0.25	80	15.5 $\pm$ 0.34	3 $\pm$ 0.28
0.5	90	17.8 $\pm$ 0.17	4.3 $\pm$ 0.31
0.75	72	12.6 $\pm$ 0.35	2.8 $\pm$ 0.17
1.0	68	9 $\pm$ 0.28	1.5 $\pm$ 0.34



Figure-3: *In vitro* rooting and hardening of *Solanum nigrum* (L.)

A) Initiation of *in vitro* roots on MS medium containing 0.5 mg/l IBA. (bar 1cm = 1.1 cm), B) Plantlet showing elongated root system. C) Hardening of plantlet in poly bag containing soil and vermiculite. D) Plantlet in field conditions.

#### Hardening and acclimatization

Rooted shoots were successfully hardened in autoclaved vermiculite and soil under high relative humidity (60-70%),

gradual decreasing air humidity and temp maintained at 26 $\pm$ 2°C after about one month, the hardened plants were transferred to polybags containing soil and vermiculite (1:1)

and were allowed to grow under nursery shade conditions. Finally these acclimatized plants were planted in field conditions with maximum survivability (Figure-3).

## Discussion

Direct regeneration from leaf is another alternative step for clonal propagation and germplasm conservation is a well established factor. The regeneration of adventitious shoots from leaf explants was dependent on both auxin and cytokinin in the medium. The presence of BA in the culture medium was found to be the key factor governing the *in vitro* response of either field grown leaves or *in vitro* grown leaves. In cytokinin alone supplemented medium after two weeks of inoculation shoot bud initiation was takes place. Induction of shoots using BAP has been well documented in *Withania somnifera* [17], *Celastrus paniculatus* [15]. In the present study at higher concentrations of cytokinin the number of shoots decreased and it promotes callusing. Similar findings were reported in *Gymnema sylvestre* [13] and *Rotula aquatica* [9]. Growth regulator requirement for direct shoot formation are generally summarized (a cytokinin to auxin ratio of 2-3:1) [10]. In field grown leaf explants the combination of BA and NAA/IAA induced maximum number of multiple shoots. Similar response with BA and IAA using leaf explants were reported in *Plumbago rosea* [11]. The effect of BA and NAA on direct shoot regeneration was reported in *Drosera* [3] and *Gysophila paniculata* [33].

In *in vitro* derived leaf explants the combination of BA-IAA/NAA promoted shoot bud regeneration and resulted in maximum number of multiple shoot induction. Promotion of shoot bud regeneration by BA either alone or in combination with IAA, has been reported in several other species such as *Withania somnifera* [2], *Spillanthus acmella* [27]. The effect of BA and NAA on direct shoot regeneration was also reported in *Becopa monnieri* [32] and *Clerodendrum inerme* [6]. Among the auxins tested (NAA, IAA and IBA) in combination with BAP, natural auxin IAA proved to be better for providing maximum number of shoots. Similar responses were reported in *Cajanus cajan* [18], *Plumbago* species [8], *Lilium* [16] and *Spillanthus acmella* [26]. In GA<sub>3</sub> supplemented medium elongated shoots were noticed. Besides promoting elongation, GA<sub>3</sub> suppressed shoot vitrification [22]. Biological activity of GA<sub>3</sub> includes promotion of cell enlargement, stem elongation (by increasing cell wall extensibility) and flowering. Gibberellins are used in plant tissue culture media to stimulate new growth and promote shoot formation and elongation. Acclimatization of regenerated plants to the external environment in the last stage of micro-propagation and its success depends on different factors as suggested by various researchers [10]. Earlier other research workers reviewed acclimatization of micro propagated plants in the green house under field conditions [24]. Rooted shoots showed the maximum percentage of survival.

Among the growth regulators used, in most of the combinations, the response of direct regeneration from leaf explants were resulted in inducing shoot regeneration directly from the leaf without forming callus. The excised leaves were successfully used for regeneration of plants and age of the excised leaves was the major factor controlling morphogenetic potential as is also true in *Cleisostoma racimeferum* [31]. There are several reports which suggest that leaves are better

source of explants for induction of multiple shoots *in vitro* [21, 29, 30 & 31]. In the present study more number of adventitious shoot formations was observed in *in vitro* grown leaf explants compared with the field grown plants.

## Conclusion

Vegetative plant parts especially leaves are desirable explants for *in vitro* improvement because of regeneration from these explants would preserve the genetic homozygosity of parent genotype. Hence in the present study excised leaves were successfully used for regeneration and *in vitro* micropropagation of *Solanum nigrum* plants.

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